Aquatic Plant Control Technical Note IC-01
September 1998

Integrated Fluridone-Fungal Pathogen Treatment of Four Submersed Plants

PURPOSE: This technical note describes an outdoor mesocosm investigation conducted to evaluate the efficacy and selectivity of the herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) and the fungal pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostazeski (*Mt*), applied alone and in combination with one another, against hydrilla (*Hydrilla verticillata* (L.f.) Royle), Eurasian watermilfoil (*Myriophyllum spicatum* L.), American pondweed (*Potamogeton nodosus* Poiret), and vallisneria (*Vallisneria americana* Michx.). Results of this research will determine the potential for integrating chemical and biological control tactics to improve the long-term management of nuisance aquatic weed species.

BACKGROUND: The goal of aquatic plant managers is to employ effective, cost-efficient, and environmentally compatible management strategies against nuisance and exotic weed species. Traditionally, these strategies have included the independent use of herbicides, biological organisms, mechanical harvesting, or habitat manipulation. Utilizing a multidisciplinary, integrated approach rather than applying a single control method may provide an alternate means for controlling nuisance plant infestations, and thus improve overall management efficiency.

The rationale for integrating control strategies is to combine the strengths of different technologies, thereby reducing inherent weaknesses of an individual technology when used alone. Integration of weed control practices has been successfully used in agro-ecosystems, but the concept has been limited in aquatic environments.

Several investigators have reported that the efficacy of some plant pathogens can be enhanced by integration with chemical herbicides (Charudattan 1986, Hoagland 1996, Netherland and Shearer 1996, Rayachhetry and Elliot 1997). In a recent review Hoagland (1996) stated that, although many pathogens have been characterized as bioherbicidal, most lack sufficient aggressiveness to overcome weed defense mechanisms to achieve adequate control. However, some herbicides and plant growth regulators can act to weaken natural plant defense systems, rendering them more susceptible to pathogen attack (Hoagland 1996).

Interactions between control agents may be antagonistic, synergistic, or additive, with additive and synergistic effects desirable for maximizing weed control. The potential advantages for implementing an integrated management strategy include increased efficacy, reduced herbicide and pathogen levels required for weed control, expanded pathogen host range, and a more economically and environmentally acceptable method of nuisance plant management (Charudattan 1986, Hoagland 1996).

Use of an integrated approach for managing the aquatic weeds waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) and Eurasian watermilfoil has been investigated by others (Charudattan 1986; Sorsa, Nordheim, and Andrews 1988). Recently, Netherland and Shearer (1996) demonstrated that combining low doses of the systemic herbicide fluridone with a fungal pathogen, *Mt*,

was effective for controlling the nuisance exotic plant hydrilla in growth chamber trials. Applying a sublethal dose of fluridone (2 µg/L) with *Mt* at rates of 100 and 200 colony forming units (CFU)/ml reduced hydrilla biomass by >90 percent and was more efficacious than applying either control agent alone.

The integrated treatment provided the benefits of rapid biomass reduction exhibited by *Mt* and the long-term prevention of hydrilla regrowth provided by fluridone. In addition, integrated treatments reduced fluridone exposure requirements by approximately 50 days, which may broaden the use of this herbicide in aquatic environments where high water exchange has limited its use in the past. Fluridone generally requires a contact time of 60 to 90 days to achieve satisfactory hydrilla control and thus has limited use in aquatic systems where high water exchange precludes long chemical-plant exposure periods (Netherland, Getsinger, and Turner 1993; Netherland and Getsinger 1995).

Herbicide selectivity can often be achieved by applying lower than recommended dosages to sensitive vegetation. Selective removal of a nuisance plant species without damaging nontarget plants is a desirable goal for many aquatic plant management situations. One advantage that may result from integrating fluridone with *Mt* is that lowering the fluridone concentration may allow increased species selectivity.

Netherland, Getsinger, and Skogerboe (1997) demonstrated in a mesocosm study that 60- and 90-day exposures of 5 µg/L fluridone were sufficient to significantly reduce Eurasian watermilfoil biomass with no effect on biomass production of nontarget species (elodea (*Elodea canadensis* Mich.), American pondweed, sago pondweed (*Potamogeton pectinatus* L.), and vallisneria), whereas higher fluridone rates (10 to 20 µg/L) injured all nontarget species. Thus, the potential exists to control the growth of noxious species with reduced rates of fluridone, without affecting desirable native species.

The objectives of this study were to verify laboratory efficacy of integrating fluridone with *Mt* for control of hydrilla, the target weed, under outdoor growing conditions and to determine the selectivity of fluridone-*Mt* treatment on other submersed plant species.

MATERIALS AND METHODS: This study was conducted in an outdoor mesocosm system at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX, which consists of large tanks (1.4 m tall by 2.6 m in diameter) that hold approximately 6,500 L of water. Each tank was individually plumbed to regulate water flow as needed and was equipped with air flow for water circulation. Further description of this mesocosm system can be found in Dick, Getsinger, and Smart (1997).

For this study, each of the 18 mesocosm tanks was divided into four equal sections, with netting to accommodate each of the four plant species. The netting allowed water flow between the divided areas but restricted plant growth to each section. Plants were grown in plastic pots (19.7 cm tall by 19.7 cm in diameter) filled with nutrient-enriched soil (one Woodace briquette (14-3-3) plus 10 g ammonium sulfate per pot). Nine pots of each plant species (three plants per pot) were placed in each tank section. Hydrilla (dioecious biotype) and Eurasian watermilfoil

were propagated from 10-cm apical cuttings and planted 4 to 5 cm into the soil. American pondweed and vallisneria were initiated from pregerminated tubers placed 4 to 5 cm into the soil.

All plants and tubers used in this study were collected from pond-grown cultures at the LAERF. Plants were allowed to establish in the mesocosm tanks for 2 months prior to herbicide-pathogen treatment. At the time of treatment, hydrilla and Eurasian watermilfoil had grown to the water surface, American pondweed had formed a surface canopy of floating leaves, and vallisneria was well established.

Treatments were applied on June 19, 1996, and included 5 µg/L fluridone, 100 and 200 CFU/ml of *Mt*, integrated treatments of 5 µg/L fluridone + 100 or 200 CFU/ml *Mt*, and untreated controls. Fluridone stock solutions were prepared from the liquid commercial formulation Sonar AS (479 g active ingredient per liter). *Mt* (isolated from hydrilla in Texas) was applied as a thick slurry of live fungal mycelium. The *Mt* inoculum was prepared as described by Shearer (1996). Both fluridone and *Mt* were applied by pouring the chemical solution and the mycelial suspension evenly over the water surface. Integrated treatments were applied simultaneously to designated tanks.

Plant biomass was harvested at 21, 42, and 84 days after treatment (DAT). At each harvest, three randomly selected pots of each plant species were removed from each mesocosm tank. Aboveground biomass was clipped at the sediment surface, washed to remove algae and debris, and dried to a constant weight at 60 °C. Plant biomass was recorded as grams dry weight per pot.

Fresh tissue samples (four samples per plant species per tank) were collected pretreatment and at each post-treatment harvest for chlorophyll analysis. The tissue selected for this procedure varied for each plant species and included 4-cm stem apices of hydrilla and Eurasian watermilfoil, floating leaves of American pondweed, and 4-cm leaf segments of vallisneria. Total chlorophyll (a and b) was measured using a DMSO extraction procedure (Hiscox and Israelstam 1979).

Water samples were collected from all fluridone-treated tanks (at 1, 2, 3, and 7 DAT, weekly thereafter through 42 DAT, and at 63 and 84 DAT) to confirm initial fluridone treatment rates and to determine herbicide dissipation. Samples were collected in 500-ml amber polyethylene bottles and frozen until analysis. Fluridone residues were detected using a high performance liquid chromatography (HPLC) procedure.[1] Residue data were subjected to linear regression procedures, and the results obtained were used to determine the half-life of fluridone under these experimental conditions.

Treatments were randomly assigned to mesocosm tanks and were replicated three times. At each sampling interval, biomass and chlorophyll data were subjected to analysis of variance and treatment means compared using Fisher's protected Least Significant Difference (LSD) test at the 0.05 level of significance.

---

1 Lilly Research Laboratory. (1980). "Method AM-AA-CA-R005-AC-755: Determination of fluridone in water by direct injection high pressure liquid chromatography," Eli Lilly and Company, Greenfield, IN.

RESULTS AND DISCUSSION: Residue analyses at 1 day after treatment (data not shown) showed that the initial target fluridone concentration (5 µg/L) was achieved in all chemically treated mesocosm tanks. Subsequent water residue data were used to determine fluridone dissipation over time. Regression analysis established that under these experimental conditions, the average half-life of fluridone in herbicide-treated tanks was 49 days (Figure 1). Fluridone dissipation was comparable to dissipation rates reported by Netherland, Getsinger, and Skogerboe (1997) under similar experimental conditions.

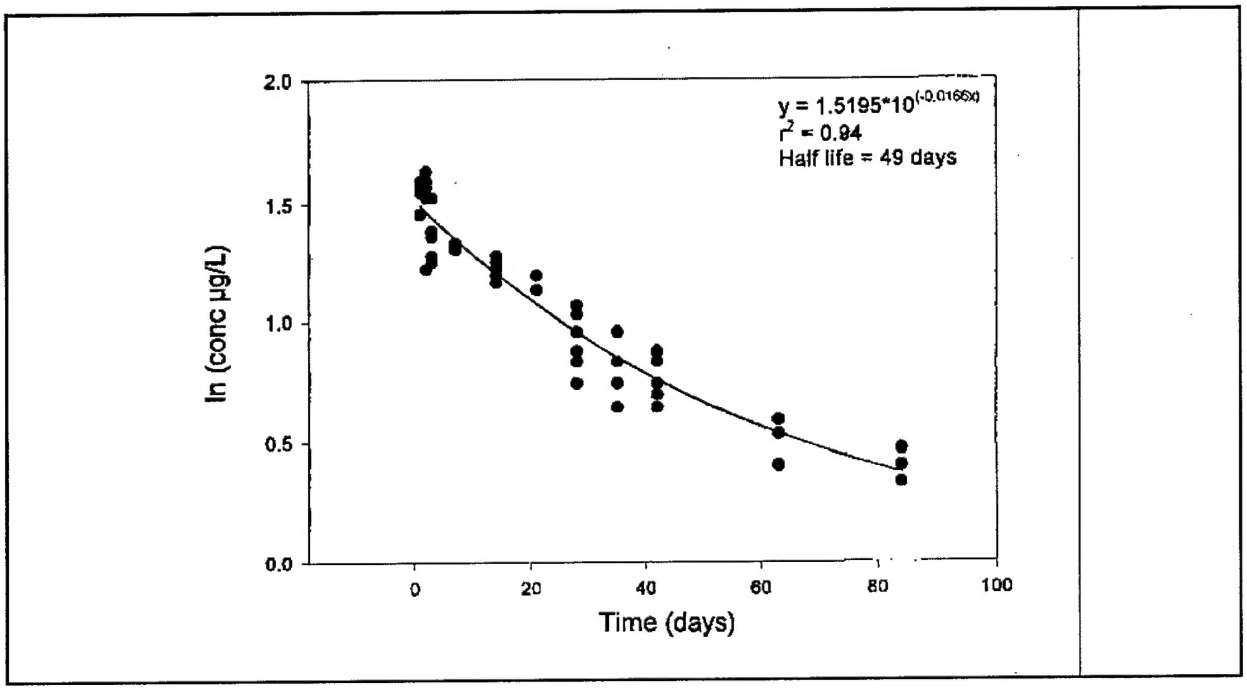

Figure 1. Dissipation of fluridone in water collected from large outdoor mesocosm tanks at Lewisville, TX. Initial treatment rate was 5 µg L^{-1}

Treatment effects on dry weight biomass varied greatly among plant species (Figure 2). The greatest response was observed on the target plant, hydrilla (Figure 2a). At 21 DAT, treatment with either fluridone alone or 200 CFU/ml *Mt* was sufficient to reduce hydrilla biomass by an average of 36 percent. However, the combined application of *Mt* plus fluridone reduced biomass up to 75 percent compared with untreated plants. By 84 DAT, the combined treatments resulted in a 93 percent reduction in hydrilla biomass. Both fluridone alone and 200 CFU/ml *Mt* reduced hydrilla biomass by 40 percent at the final harvest. Statistically, there were no differences between the two rates of *Mt* or between fluridone alone and *Mt* at 200 CFU/ml on hydrilla.

Characteristic injury symptoms of fluridone and *Mt* were observed on hydrilla. Successful fungal infection was noted on all *Mt*-treated tanks 10 DAT and was identified by leaf tip chlorosis and stem defoliation. Although biomass was not significantly different between the two rates of *Mt*, disease symptoms were visibly more abundant on tanks treated with the higher than the lower rate of *Mt*. At the first post-treatment harvest, new and healthy hydrilla growth (lateral shoots from viable stems) also was present in all tanks treated with *Mt* by itself. Fluridone effects on hydrilla (pink stem coloration and bleached leaves on new tissues) were observed 21 DAT.

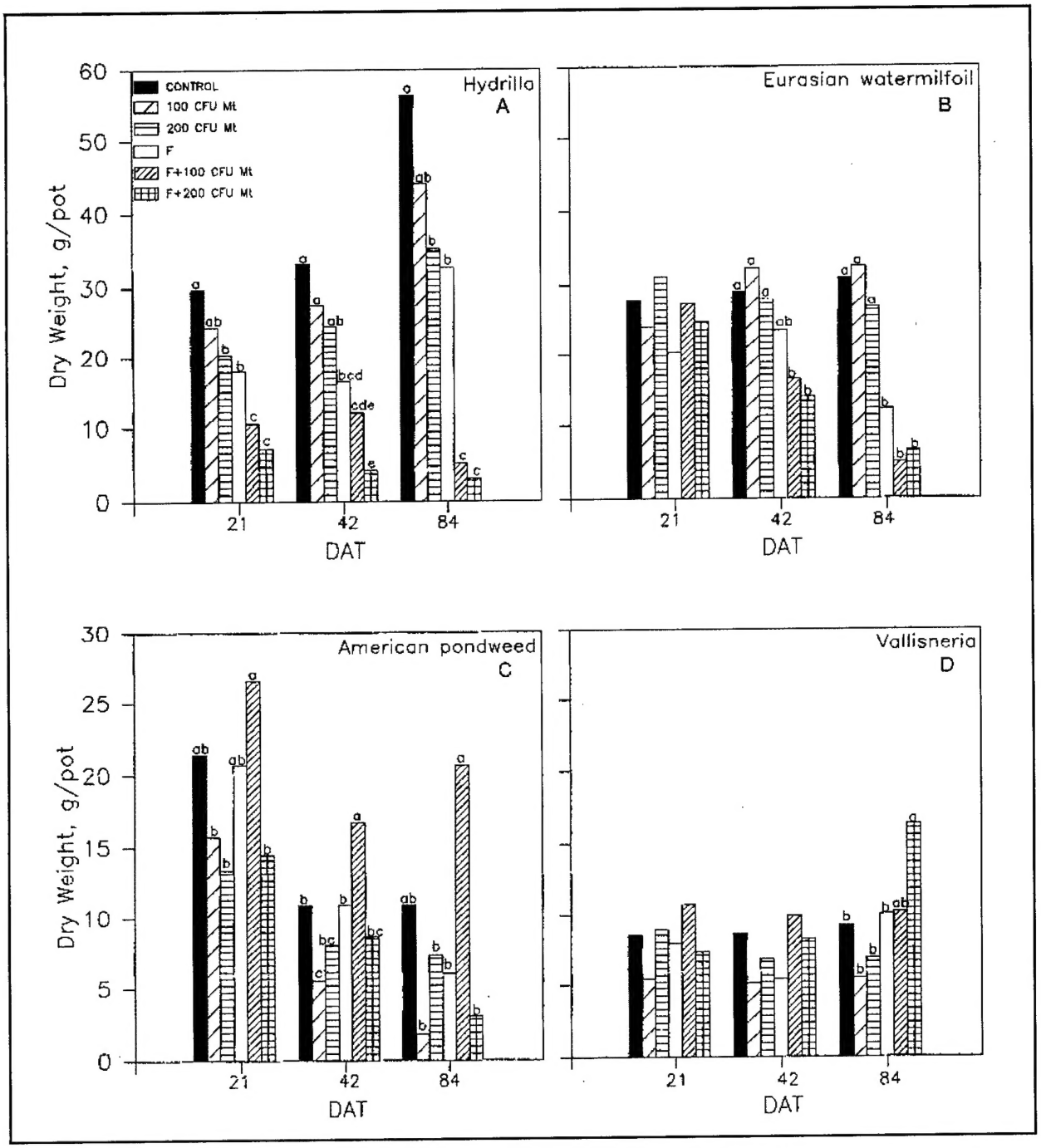

Figure 2. Mean dry weight biomass of hydrilla (A), Eurasian watermilfoil (B), American pondweed (C), and vallisneria (D) at 21, 42, and 84 days after treatment (DAT) following application of *Mt* at 100 and 200 colony forming units (CFU) per milliliter, fluridone (F = 5 µg/L fluridone), and integrated treatments of fluridone + *Mt*. Within each sample time, means followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected LSD test

Fluridone, but not *Mt*, symptomology was also observed on Eurasian watermilfoil. Neither vallisneria nor American pondweed displayed visible symptoms of fungal infection or fluridone leaf bleaching.

Although Eurasian watermilfoil was not the target plant in this study, treatment with fluridone alone and fluridone plus either 100 or 200 CFU/ml *Mt* reduced Eurasian watermilfoil biomass by 75 percent at 84 DAT (Figure 2b). Unlike the synergistic effect observed on hydrilla, the response on Eurasian watermilfoil was likely due to fluridone itself, as there were no statistical differences between treatments of fluridone alone and those integrated with *Mt*. The fact that effects on biomass were not observed until late in the study (42 DAT) further implies fluridone activity as the main source of efficacy.

Fluridone is a slow-acting herbicide compared to the quick infection response observed with *Mt* (Netherland and Shearer 1996). Results are consistent with other outdoor mesocosm studies in which fluridone at a rate of 5 µg/L was sufficient to reduce Eurasian watermilfoil biomass (Netherland, Getsinger, and Skogerboe 1997). Strains of *Mt* (other than that used in this study) have been isolated for activity on Eurasian watermilfoil and were found to be effective in greenhouse trials (Gunner and others 1990). Combining milfoil-specific strains of *Mt* with fluridone may have potential as an integrated approach for management of Eurasian watermilfoil, and should be evaluated.

Nontarget species were less affected by fluridone and *Mt*. Compared with untreated plants, none of the treatments inhibited biomass of American pondweed at 21 DAT (Figure 2c). Results were variable at subsequent harvests. For example, *Mt* at 100 CFU/ml significantly reduced biomass by 50 percent 42 DAT, while treatment with fluridone + 100 CFU/ml *Mt* resulted in a significant increase (35 percent) in biomass. By the end of the study, none of the treatments was statistically different from controls; however, fluridone + 100 CFU/ml *Mt* showed significantly higher biomass when compared with other fluridone or *Mt* treatments.

Some of the observed variation in biomass data can be attributed to insect damage. At 21 DAT, floating leaves of American pondweed had been severely decimated by an unidentified species of whitefly (*Trialeurodes* sp.) and a common aquatic insect identified as the larva of the waterlily leafcutter (*Synclita obliteralis* (Walker)). Infestation was not evenly distributed among tanks (some tanks were not infested at all) and may account for the variability in biomass data observed on this plant species. American pondweed in two of the three replicate tanks treated with fluridone + 100 CFU/ml *Mt* did not show insect damage, which may explain the high biomass levels recorded for this treatment.

Vallisneria biomass was not inhibited by any of the applied treatments (Figure 2d). No statistical differences among treatments were noted at 21 and 42 DAT, and by the final harvest, only fluridone + 200 CFU/ml *Mt* was significantly different from untreated plants. For reasons unknown, this treatment showed a 44 percent increase in biomass compared with untreated plants.

With the exception of American pondweed, all treatments that included fluridone significantly reduced total chlorophyll content in sampled tissues (Table 1). Hydrilla was most sensitive, with chlorophyll decreases of >70 percent measured at 21 DAT and a >50 percent decrease recorded

thereafter. For Eurasian watermilfoil, chlorophyll content in fluridone-treated plants was 32 to 39 percent less than that of untreated plants throughout the study. Initially, vallisneria showed reduced leaf chlorophyll (by 29 percent at 21 DAT). However, at 84 DAT there were no differences among treatments, indicating plant recovery. For all plant species, *Mt* alone did not affect total chlorophyll at the times sampled. Netherland and Shearer (1996) showed reduced chlorophyll content in hydrilla at 7 and 14 DAT with 100 and 200 CFU/ml *Mt*, but the effects dissipated by 28 DAT.

Table 1. Effect of Fluridone, *Mt*, and Fluridone + *Mt* Treatments on Total Chlorophyll Content of Four Submersed Plant Species

Species	Treatment (µg/L + CFU)[1]	Total Chlorophyll Content (mg g-1 fr wt)			
			Days after Treatment[2]		
		Pretreatment	21 DAT	42 DAT	84 DAT
Hydrilla	Untreated	1.17	1.11 a	1.09 a	1.12 a
	0 + 100	1.04	0.95 a	1.15 a	1.14 a
	0 + 200	1.02	0.97 a	1.03 a	1.22 a
	5 + 0	1.21	0.20 c	0.50 b	0.44 b
	5 + 100	1.14	0.30 bc	0.44 b	0.54 b
	5 + 200	1.16	0.39 b	0.52 b	0.56 b
	(LSD)	NS	(0.19)	(0.25)	(0.23)
E. watermilfoil	Untreated	1.44	1.56 a	1.73 a	1.35 a
	0 + 100	1.58	1.53 a	1.70 a	1.51 a
	0 + 200	1.47	1.65 a	1.77 a	1.49 a
	5 + 0	1.40	1.05 b	1.03 b	0.98 b
	5 + 100	1.45	1.09 b	1.00 b	0.81 b
	5 + 200	1.50	1.06 b	1.14 b	0.92 b
	(LSD)	NS	(0.25)	(0.20)	(0.26)
American pondweed	Untreated	1.42	1.10	1.40	1.43 b
	0 + 100	1.53	0.86	1.30	1.41 b
	0 + 200	1.74	0.97	1.40	1.51 b
	5 + 0	1.54	1.19	1.39	1.32 b
	5 + 100	1.70	1.11	1.30	1.27 b
	5 + 200	1.63	0.95	1.15	1.83
	(LSD)	NS	NS	NS	(0.32)
Vallisneria	Untreated	0.86	0.78 b	0.85 ab	0.68
	0 + 100	0.86	0.97 a	0.78 abc	1.35
	0 + 200	0.87	0.78 b	0.93 a	0.78
	5 + 0	0.87	0.52 c	0.66 bc	0.50
	5 + 100	0.92	0.62 c	0.64 bc	0.46
	5 + 200	0.74	0.51 c	0.58 c	0.63
	(LSD)	NS	(0.13)	(0.22)	NS

Note: Within columns, means followed by different letters are significantly different (Least Significant Difference, $P \leq 0.05$); NS = not significant.
[1] Fluridone concentration (expressed in µg/L) plus colony forming units of *Mycoleptodiscus terrestris.*

The results of this study confirm those observed in growth chamber studies by Netherland and Shearer (1996). For hydrilla, a beneficial synergistic interaction was observed with combined applications of 5 µg/L fluridone with either 100 or 200 CFU/ml *Mt*. Neither control agent alone provided adequate hydrilla control. For Eurasian watermilfoil, 5 µg/L fluridone was sufficient to significantly reduce biomass, which was consistent with reports that maintenance of low doses of fluridone over time can significantly inhibit biomass production (Netherland, Getsinger, and Skogerboe 1997). There was no advantage to integrating fluridone with *Mt* on Eurasian watermilfoil. At the rates applied, the strain of *Mt* utilized in this study was ineffective on

Eurasian watermilfoil. Other strains of *Mt* have been isolated for pathogenicity on this plant species and may be potential candidates for integrating with fluridone.

The desired level of selectivity was achieved with the integrated treatments applied in this study. Biomass of American pondweed and vallisneria was not severely impacted by treatment rates sufficient to control the target species, hydrilla. The results demonstrated that by integrating fluridone and *Mt*, a low herbicide rate that reduced the likelihood of chemical damage to nontarget species could be used. The potential for selectivity gives further merit to the concept of integrated weed management.

FUTURE WORK: Future research will focus on larger scale field testing of fluridone-*Mt* treatments for controlling hydrilla, as well as evaluating other potential herbicide-pathogen combinations for aquatic plant management. Development of a granular *Mt* formulation to provide an easier and more uniform means of application has also been initiated.

Initial field tests were conducted in June 1997 in nine small ponds located at the Center for Aquatic Plants in Gainesville, FL. These ponds (0.15 acre-foot) were nearly 100 percent covered with hydrilla and represent situations where fluridone injury is often delayed due to the lack of active plant growth in a dense canopy of hydrilla. Treatments included fluridone alone (15 µg/L), *Mt* alone (150 CFU/ml), fluridone + *Mt* (15 µg/L + 150 CFU/ml), and fluridone plus the contact herbicide copper (15 µg/L + 250 µg/L). Hydrilla biomass and chlorophyll content as well as water quality changes were monitored at 0, 6, and 12 weeks after treatment. Results of this study and additional pond studies, to be conducted at the Lewisville Aquatic Ecosystem Research Facility, will be discussed in future technical notes.

POINTS OF CONTACT: For additional information contact the authors, Ms. Linda S. Nelson, (601) 634-2656, *nelsonl@mail.wes.army.mil,* Dr. Judy F. Shearer, (601) 634-2516, *shearej@mail.wes.army.mil,* and Mr. Michael D. Netherland, (601) 634-3889, *netherm1@mail.wes.army.mil,* or the manager of the Aquatic Plant Control Research Program, Dr. John W. Barko, (601) 634-3654, *barkoj@mail.wes.army.mil.* This technical note should be cited as follows:

> U.S. Army Engineer Waterways Experiment Station. (1998). "Integrated Fluridone-Fungal Pathogen Treatment of Four Submersed Plants," Aquatic Plant Control Technical Note IC-01, Vicksburg, MS.

REFERENCES:

Charudattan, R. (1986). "Integrated control of waterhyacinth (*Eichhornia crassipes*) with a pathogen, insects, and herbicides," *Weed Science* 34 (Suppl. 1), 26-30.

Dick, G. O., Getsinger, K. D., and Smart, R. M. (1997). "Outdoor mesocosm system for evaluating aquatic herbicides," Miscellaneous Paper A-97-3, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Gunner, H. B., Limpa-amara, Y., Bouchard, B. S., Weilerstein, P. J., and Taylor, M. E. (1990). "Microbiological control of Eurasian watermilfoil," Technical Report A-90-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Hiscox, J. D., and Israelstam, G. (1979). "A method for the extraction of chlorophyll from leaf tissue without leaf maceration," *Canadian Journal of Botany* 57, 1332-34.

Hoagland, R. E. (1996). "Chemical interactions with bioherbicides to improve efficacy," *Weed Technology* 10, 651-74.

Netherland, M. D., and Getsinger, K. D. (1995). "Potential control of hydrilla and Eurasian watermilfoil under various fluridone half-life scenarios," *Journal of Aquatic Plant Management* 33, 36-42.

Netherland, M. D., Getsinger, K.D., and Skogerboe, J. D. (1997). "Mesocosm evaluation of the species-selective potential of fluridone," *Journal of Aquatic Plant Management* 35, 41-50.

Netherland, M. D., Getsinger, K. D., and Turner, E. G. (1993). "Fluridone concentration and exposure time requirements for control of Eurasian watermilfoil and hydrilla," *Journal of Aquatic Plant Management* 31, 189-94.

Netherland, M. D., and Shearer, J. F. (1996). "Integrated use of fluridone and a fungal pathogen for control of hydrilla," *Journal of Aquatic Plant Management* 33, 4-8.

Rayachhetry, M. B., and Elliot, M. L. (1997). "Evaluation of fungus-chemical compatibility for melaleuca (*Melaleuca quinquenervia*) control," *Weed Technology* 11, 64-69.

Shearer, J. F. (1996). "Field and laboratory studies of the fungus *Mycoleptodiscus terrestris* as a potential agent for management of the submersed aquatic macrophyte *Hydrilla verticillata*," Technical Report A-96-3, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Sorsa, K. K., Nordheim, E. V., and Andrews, J. H. (1988). "Integrated control of Eurasian watermilfoil, *Myriophyllum spicatum*, by a fungal pathogen and a herbicide," *Journal of Aquatic Plant Management* 26, 12-17.